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Validated method for determination of degradation impurity of Noscapine HCl by HPLC

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ABSTRACT

A simple, rapid, and sensitive RP-HPLC method was developed and validated for the simultaneous determination of degradation impurity of Noscapine HCl. The method was developed to determine Papaverine in Noscapine Hydrochloride. Separation was achieved within 45 minutes on a Waters Sunfire, C18, 250 x 4.6 mm, 5 µ particle size column using mobile phase consisting of 1-octane sulfonic acid buffer pH-3.0 and Acetonitrile. Gradient flow mode with a flow rate of 0.8 mL/min and detection was carried out at 260 nm. The column temperature was 45°C while sample temperature was 25°C. A complete validation procedure was carried out. There was good resolution observed between Noscapine and Papaverine Peak which is about 2.8. The proposed method exhibited excellent linearity over the concentration ranges of 1.2 µg/mL to 6.0 µg/mL for Papaverine and Noscapine. The proposed method was applied for bulk drug of Noscapine HCl for accuracy study with recovery of 101.26% to 104.52%.

Keywords: Noscapine, HPLC, Validation, Papaverine and Degradation.

INTRODUCTION

The reference of Noscapine HCl is not found in majority of pharmaceutical and chemistry books. Today majority of marketed dosage forms are of Noscapine HCl eg., Coscopine. There is one related substance (Impurity) of Noscapine HCl mentioned in United states Pharmacopoeia e.g., Papaverine. Papaverine can create polymorphic ventricular tachycardia, constipation, interference with suphobromoptalein retention test, increased transamine levels, increased alkaline phosphatase levels, somnolence and vertigo. Therefore, the amount of Papaverine in Noscapine HCl should be controlled during manufacturing process of Noscapine HCl as an active pharmaceutical ingredient. After doing thorough literature review it was found that analytical methods for estimation of related substances in Noscapine HCl bulk substances and in dosage forms are not available in majority. Most of the official methods available in USP are by Thin layer technique for estimation of related substances in Antitusive drug. The TLC method is not accurate and precise as compared to HPLC/UPLC technique. Reviewing the scientific literature revealed a ATR-FT-IR and FT-Raman spectroscopy metod is developed for determination of *papaver somnifer* which is not as accurate as HPLC[9].

There is a scope to develop a fast, cost effective, precise and accurate HPLC/UPLC technique method to determine the Papaverine content in Noscapine HCl drug API. This method will help all the pharmaceutical industries to adopt the method for their product development and routine analysis of Antitusive drug, which will be cheap and fast.

MATERIALS AND METHODS

Instruments:
Separation was performed on Waters HPLC system 2695 model with empower pro 3.0 software. The equipment was equipped with Ultra violet as well as photo diode array detector. Injection loop for the equipment was of 100 µL. The HPLC was having pump containing quaternary channel with online degasser mode.

Materials:
Noscapine HCl and Papaverine were procured from Biological E. Ltd., Hyderabad as a gift sample.

Reagents:
All solvents used were of HPLC grade and all chemicals were of analytical grade. High purity Milli-Q water filtered through 0.45 µ size membrane filter was used throughout the study. Acetonitrile was obtained from Merck chemicals, while 1-octane sulphonic acid and *ortho* phosphoric acid was obtained from Rankem.

Preparation of Solutions
Standard solution
Stock solution of Noscapine HCl and Papverine of concentration of 200 µg/mL were prepared individually in 0.1 N HCl. The standard stock solution was further diluted with 0.1 N HCl to get 2 µg/mL of standard solution. Standard solution was found to be stable for 24 hours.

LOQ solution
Standard solution was further diluted with 0.1 N HCl to get 1.2 µg/mL of LOQ solution.

Sample Solution
Noscapine HCl API was dissolved in 0.1 N HCl to get 200 µg/mL of sample solution.

Stressed degradation samples
Acid degradation sample:
10 mg of Noscapine hydrochloride was weighed and transferred to 50 mL volumetric flask. 1 mL of 1 N HCl was added to the flask and was kept at room temperature for 1 hour. The solution was neutralized by adding 1 mL of 1 N NaOH. The volume was made up with 0.1 N HCl.

Base degradation sample:
10 mg of Noscapine hydrochloride was weighed and transferred to 50 mL volumetric flask. 1 mL of 1 N NaOH was added to the flask and was kept at room temperature for 1 hour. The solution was neutralized by adding 1 mL of 1 N HCl. The volume was made up with 0.1 N HCl.

Peroxide degradation sample:
10 mg of Noscapine hydrochloride was weighed and transferred to 50 mL volumetric flask. 1 mL of 30% H_2O_2 was added to the flask and was kept at room temperature for 1 hour. The volume was made up with o.1 N HCL.

Experimental
Separation was performed on a Waters Sunfire, C18, 250 x 4.6 mm, 5 µ particle size column using mobile phase consisting of 1-octane sulfonic acid buffer pH-3.0 and Acetonitrile.

Preparation of Buffer pH-3.0: 1.1 g of 1-octane sulfonic acid sodium salt (anhydrous) was weighed and transferred to 1000 mL of Milli-Q water and mixed well. The pH of the solution was adjusted to 3.0 ± 0.05 with 2% ortho phosphoric acid solution.

The gradient pump flow mode was used in which mobile phase A consisted of 1-octane sulfonic acid buffer pH-3.0 were as mobile phase B was 1-octane sulfonic acid buffer pH-3.0: Acetonitrile (30:70 v/v). The separation was carried out at 0.8 mL/min flow rate with injection volume of 30 µL. Detection wavelength was 260 nm on PDA/UV detector. The column temperature was 45°C while sample temperature was 25°C.

Gradient programme:

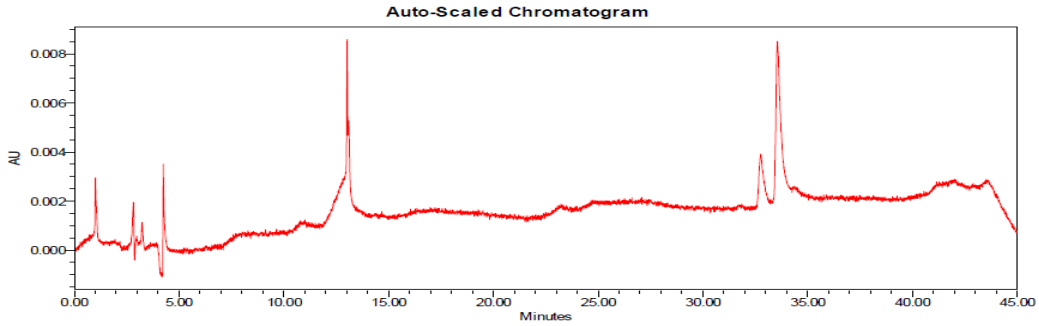
Time	% of Mobile phase-A	% of Mobile phase-B
0	80.0	20.0
5	80.0	20.0
15	65.0	35.0
35	45.0	55.0
40	32.5	67.5
41	80.0	80.0
45	80.0	20.0

RESULTS AND DISCUSSION

Specificity:

Specificity was carried out by checking the interference of diluents and by carrying out forced degradation. There was no blank peak interfering at the retention time of Noscapine and Papaverine.

Figure 1. Chromatogram of Blank solution



Forced degradation was carried out by stressing the sample solution at Acid, Base and Peroxide condition. The maximum degradation was achieved at peroxide stress condition of about 8.59 %. The peak was pure in all the degraded samples and mass balance was also found to be matching.

Figure 2. Chromatogram for Peroxide stressed sample

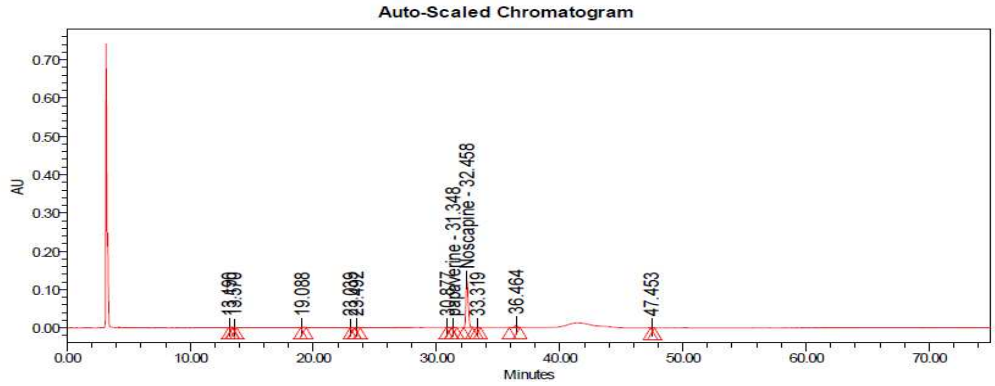


Table 1. Forced degradation data for stressed samples

Sample	Assay of Noscapine (%)	Total Impurities (%)	Total Mass balance (%)	Peak purity
As such sample	97.73	1.47	99.20	Passing
Acid degraded sample	96.80	1.81	98.61	Passing
Base degraded sample	95.53	4.25	99.78	Passing
Peroxide degraded sample	88.25	8.59	96.84	Passing

Linearity

Linearity was carried out carried out from LOQ level to 150 % of the standard concentration for Noscapine HCl and Papaverine. The r^2 value for Noscapine was 0.999 and for Papaverine it was 0.999.

Figure 3. Linearity plot for Papaverine

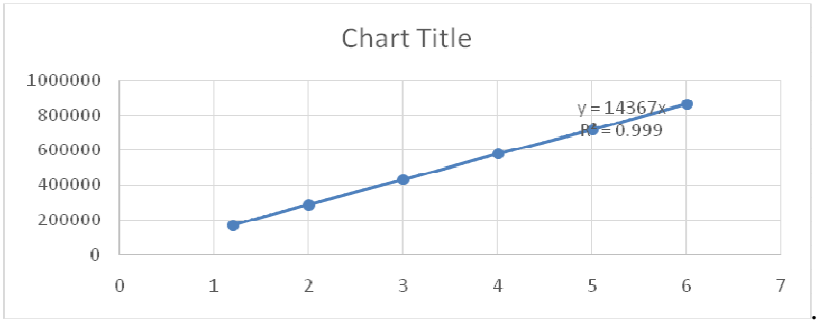
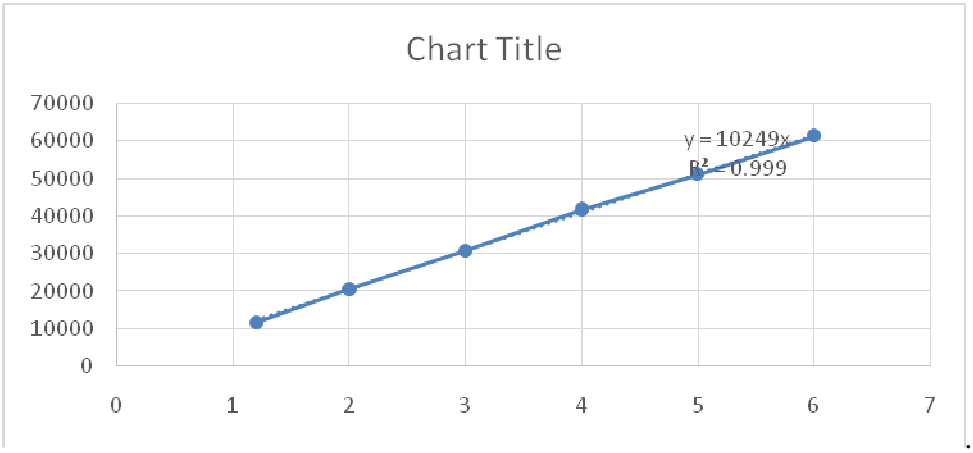


Figure 4. Linearity plot for Noscapine HCl



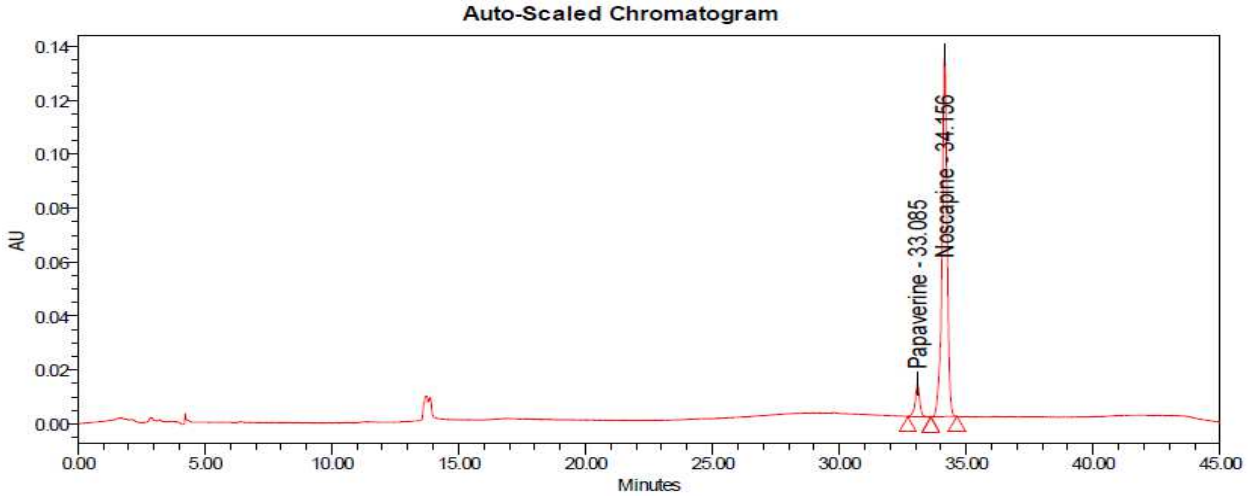
Accuracy

Accuracy was carried out by spiking the LOQ, 50%, 100% and 150% level of Papaverine standard solution into the sample solution. The recovery was found to from 101.26% to 104.52%.

Table 2. Results of recovery for accuracy study

Sr. No	Sample	Concentration (µg/mL)	Average Recovery (%)
1.	LOQ level	1.2	104.52
2.	50% Level	2	101.58
3.	100% Level	4	101.26
4.	150% Level	6	101.55

Figure 5. Chromatogram of Precision sample



Precision
Precision study was carried out by spiking the Papaverine solution at LOQ level in the sample solution. The % RSD for six samples for the recovery of Papaverine was found to be 1.81%.

Solution stability
Solution stability was carried at room temperature for sample as well as for standard solution at 24 hours. The sample solution as well as standard solution was found to be stable for 24 hours at room temperature.

CONCLUSION

A fast, simple and accurate stability-indicating HPLC method has been developed for the identification of impurities and degradants of Noscapine HCl in active pharmaceutical ingredient. The method allows identification, quantitative analysis, and purity assessment to be performed simultaneously. The proposed method appears to be suitable for quality control laboratories, where economy and time-saving are essential.

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